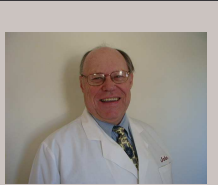


Spore News

Volume 2, Number 3
September 2005



John R. Gillis, Ph.D.
President

What does the Survival/Kill Calculation on the Certificate of Analysis Really Indicate?

We receive frequent questions from customers regarding the kill time that appears on the certificate. The impression it gives is that the kill time is “too long” and the biological indicators are too resistant. This is a misconception. Let’s look at the facts:

The Survival/Kill calculations were initially used when the survivor curve method was the primary means of calculating the D-value. The survivor curve D-value method measures the number of surviving spores after a sterilization insult. It is not able to detect less than 30 surviving spores. It does not have the ability to detect a “sterile” end point. The D-value is determined using the slope of the log linear regression plot of the reduced spore numbers compared to exposure time. Therefore, the survival/kill calculations were used to estimate the maximum time a spore would survive and the minimum kill time when all spores are killed. This approach is nearly 50 years old. Microbiology lab environments and equipment have changed significantly over the years. The kill time estimate is very conservative because the test was historically performed using 100 BI units. These were cultured in the “open” lab environment. A test was acceptable if only 99 units out of 100 were killed. The one positive sample was considered “lab error” or accidental contamination. Today we have Class 100 laminar flow work stations or biosafety cabinets to work in which are far more reliable than open bench tops. Therefore, from a very practical approach this kill value is extremely conservative.

The fraction negative D-value approach became popular about 35 years ago. The Stumbo, Murphy, Cochran method was commonly used. This D-value method measured the quantal zone for fractional survivors only. It is not possible to use a “0” survivor or “0” kill data point with this equation. Therefore, the application of this survival/kill calculation was still of value.

Today the standards require manufacturers of BI’s to determine the D-value using two methods: 1) Survivor Curve; and 2) Limited Holcomb, Spearman, Karber (fraction negative method). This accomplishes many things. The Survivor Curve will demonstrate the linearity of spore lethality. The Limited Holcomb,

Spearman, Karber method requires an empirical survival data point and an empirical kill data point as well as the quantal zone indicating a mean time to sterility. Using these two methods you get it all. The survival/kill calculation as outlined by the standards has lost its value. It would be best if this calculation were removed from all regulatory standards or note that it only has value if the Limited Holcomb, Spearman, Karber (LHSK) data is not available.

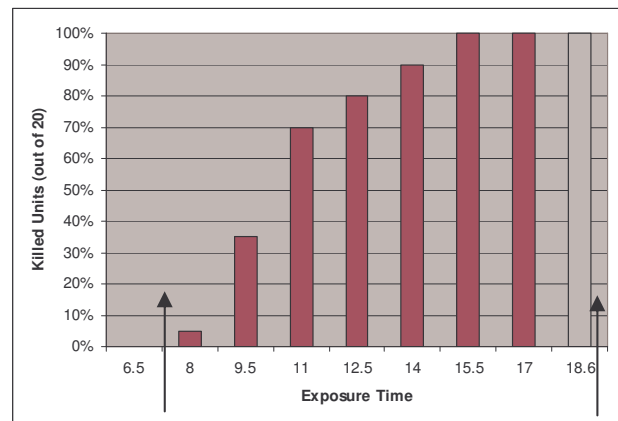
$$\begin{aligned} \text{Survival time} &= [(\log N_0 - 2^{(1)}) \times \text{Dvalue}] \\ \text{Kill time} &= [(\log N_0 + 4^{(2)}) \times \text{Dvalue}] \end{aligned}$$

⁽¹⁾This is the log₁₀ number of the number of samples required for the test (e.g.100)

⁽²⁾This is the log₁₀ number of the samples required for the test (e.g. 100) plus the possibility of having one accidental contamination out of 100 samples tested.

LHSK Data:

121° C Exposure Time (Min.)	# of Sterile Units/20
6.5 Empirical Survival Time	0/20
8.0	1/20
9.5	7/20
11.0	14/20
12.5	16/20
14.0	18/20
15.5 Empirical Kill Time	20/20
17.0	20/20



Survival Time 6.6 min
Calculation

Kill Time 18.6 min
Calculation

Exposure interval \cong 75% of Dvalue

Total units tested = 160

D-value = 2.0 minutes

Population = 2.0×10^5 spores/unit

$$\begin{aligned} \text{Survival time} &= [(\log_{10} 2.0 \times 10^5 - 2) \times \text{Dvalue}] \\ &= [(5.30 - 2) \times 2.0 \text{ minutes}] \\ &= 3.30 \times 2 \\ &= 6.6 \text{ minutes} \end{aligned}$$

Note: The empirical data indicates that the actual survival time is between 6.5 and 8.0 minutes. Therefore, the survival time calculation provides a reasonable agreement with the empirical data. The actual survival time is likely closer to 8 minutes since only one sterile unit out of 20 was observed.

$$\begin{aligned}
\text{Kill time} &= [(\log_{10} 2.0 \times 10^5 + 4) \times D\text{value}] \\
&= [(5.30 + 4) \times 2.0 \text{ minutes}] \\
&= 9.30 \times 2.0 \\
&= 18.6 \text{ minutes}
\end{aligned}$$

Note: The empirical data illustrates that this kill time is between 14.0 minutes and 15.5 minutes, probably closer to 15 minutes. The calculation overstates the kill time for BI's by 3.1 minutes to 4.6 minutes. Thus, indicating that there is a chance to observe a surviving BI well beyond the actual kill time documented.

Until the standards change, manufacturers will continue to supply this required information. It would be much more useful if manufacturers were to include on their label the empirical survival and kill times observed by the Limited Holcomb, Spearman, Karber method. This is an opportunity to contact your local representative to the ISO committee, ISO/TC 198 WG4 and the USP Expert Advisory Committee for Microbiology to propose appropriate changes to eliminate this required calculation and propose that the actual test data be presented.

Please email us with topics you would like to see addressed in “Spore News”.

